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Evaluation of soybean for resistance to soybean rust in Vietnam☆

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ABSTRACT

Soybean rust, caused by *Phakopsora pachyrhizi* Sydow, is a severe foliar disease of soybean [*Glycine max* (L.) Merr.] that occurs throughout most soybean producing regions of the world. The objective of this research was to evaluate selected soybean genotypes for resistance to soybean rust in Vietnam. Five field experiments in Vietnam were completed from 2006 to 2009. The area-under-the-disease-progress-curve (AUDPC) was calculated for each soybean genotype based on four disease assessments taken during the reproductive growth stages. AUDPC units among soybean genotypes in each experiment differed ($P < 0.05$). Over the five experiments, the resistant check DT 2000 was most often the genotype with the lowest AUDPC units while the sources of rust resistance (*Rpp1-5*) did not always have low AUDPC units in each experiment, although PI 230970 (*Rpp2*) appeared to be more stable. A few genotypes with non-characterized genes for resistance, such as PI 398998, PI 437323, and PI 549017, had the lowest AUDPC units in at least one of the experiments. These genetic resources may be useful for host plant resistance studies and breeding soybeans for rust resistance in Vietnam and other locations like Brazil and the United States that have more recently been inundated with soybean rust. A significant ($P < 0.001$) experiment × genotype interaction was found when the AUDPC data of 14 soybean genotypes tested in Experiments 1, 2, and 3 were combined and analyzed. This result indicates the potential importance of changing fungal races and/or biotypes that occur in the rust population.

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1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an important source of oil for human consumption and industry, and a protein-rich meal for livestock feed. Among diseases that threaten this crop, soybean rust is one of the most severe (Hartman et al., 1999). Favorable environmental conditions for disease development result in substantial yield losses (Hartman et al., 1991; Yorinori et al., 2005). The causal fungus *Phakopsora pachyrhizi* Sydow was documented in 1903 as *Uredo sojae* from rust-infected leaves of *G. max* subsp. *soja* or wild soybean in Japan (Hennings, 1903), and has since been reported in many countries throughout the Eastern Hemisphere (Hartman et al., 1999). Soybean rust was first detected in the U.S. in Hawaii in 1994 (Killgore and Heu, 1994) and in the continental U.S. in 2004 (Schneider et al., 2005).

Deployment of resistant soybean cultivars is the preferred management method because it is more economical and impacts the environment less than other methods. Resistance expression in soybean basically ranges from apparent immunity (no visible rust lesions) to red-brown (RB) lesions with limited sporulation, however variations on the typical RB reaction type have been observed in some soybean genotype-rust pathotype interactions. The completely susceptible reaction is characterized by tan lesions (TAN) with profuse sporulation (Bromfield, 1984). Seven dominant resistance genes at five loci have been identified in soybean (Calvo et al., 2008; Chakraborty et al., 2009; Garcia et al., 2008; Hartwig, 1986; Hartwig and Bromfield, 1983; Monteros et al., 2007) that control pathotype-specific resistance. When challenged with specific *P. pachyrhizi* isolates, some of these resistant sources produce a susceptible reaction (Bonde et al., 2006; Paul and Hartman, 2009; Pham et al., 2009).

☆ Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1
Soybean genotypes tested for resistance to soybean rust in five field experiments at the Vietnam Agriculture Science Institute (2005) and the Plant Protection Research Institute (2006–2009), Hanoi, Vietnam.

Genotype	Maturity group	Exp.	Genotype	Maturity group	Exp.
Bragg	VII	1, 3	PI 427241	VI	1, 2, 3
Cao Bang U8352		1, 2, 3	PI 429329	VII	1, 3
Cook	VIII	1	PI 434973A	X	1
DT 12	III	1	PI 437323	III	1, 3, 4
DT 2000	IV	1, 2, 3, 4	PI 459025B	X	1, 3, 4, 5
Essex	V	1	PI 459025F	IX	1, 2, 3
Ina	IV	1	PI 462312	VIII	1, 2, 3, 5
Jupiter-R	IX	1, 2, 3	PI 471904	IX	5
Lee 74	VI	1	PI 506695	VI	5
Pana	III	1, 2, 3	PI 506947	IV	5
PI 068494	III	1, 3	PI 508269	IV	1
PI 081765	I	5	PI 561356	V	5
PI 083881	IV	1	PI 518759	V	1, 3
PI 085089	V	1, 3	PI 547875	III	1
PI 088452	IV	1, 3	PI 547878	III	1
PI 091730	III	1	PI 548178	III	1
PI 092560	III	1	PI 548463	VI	1, 2, 3
PI 164885	VIII	1, 3	PI 548484	VI	1, 2, 3
PI 165914	VII	1	PI 549017	IV	1, 3, 4
PI 189402	VII	1, 2, 3	PI 561287A	IV	1, 2, 3
PI 200456	VIII	5	PI 561287B	IV	1, 3
PI 200478	III	5	PI 561377	II	5
PI 200492	VII	1, 2, 3, 5	PI 561381	V	1
PI 200526	VIII	5	PI 567024	VIII	5
PI 203398	VIII	5	PI 567031B	VIII	5
PI 206258	VIII	1, 3	PI 567034	VIII	5
PI 208437	VII	1, 3, 4	PI 567046A	VIII	5
PI 230970	VII	1, 2, 3, 4, 5	PI 567104B	IX	5
PI 240667A	IX	1, 3	PI 567565	IV	1, 3, 4
PI 243524	IV	1	PI 587866	VI	5
PI 319525	VI	1, 3	PI 594172A	VII	1, 2, 3, 5
PI 340898A	IX	1	PI 594250	IV	1
PI 379618	V	5	PI 594538A	IX	1, 3, 4, 5
PI 379621	VI	5	PI 594754	IX	5
PI 385942	IV	1	PI 594767A	IX	5
PI 398998	VI	1, 2, 3, 4	PI 605773	VII	5
PI 407730	III	1, 3	PI 605791A	VI	5
PI 417088	V	1	PI 605829	V	5
PI 417089A	IX	1, 3, 5	PI 605885B	V	5
PI 417115	VII	5	PI 606405	V	5
PI 417116	VII	5	PI 615437	VI	5
PI 417120	VIII	5	PI 628859	VIII	1, 3
PI 417125	VIII	5	Rend	IV	1, 3
PI 417317	VIII	1, 3	Vang Ha Giang		1, 3
PI 423972	IX	1, 4	Williams 82	III	1, 2, 3, 4, 5
PI 424456	VI	1			

Recently, over 16,000 soybean accessions from the USDA-ARS Germplasm Collection, Urbana, IL, were evaluated for resistance to a mixture of four *P. pachyrhizi* isolates in seedling evaluations, and 805 accessions with low severity or reddish-brown lesions were identified (Miles et al., 2006). Subsets of these 805 accessions were field tested in Nigeria and Paraguay, and high levels of field resistance were identified in some accessions (Miles et al., 2008; Twizeyimana et al., 2008). The objective of this research was to evaluate selected soybean genotypes for resistance to soybean rust in Vietnam.

2. Materials and methods

2.1. Location of the study

Five field experiments were conducted at the Vietnam Agricultural Science Institute (VASI) in 2005 and at the Plant Protection Research Institute (PPRI) during 2006–2009; both are research stations of the Ministry of Agriculture and Rural Development (MARD) based in Hanoi, Vietnam. The latitude and longitude for PPRI is 21°04′08.14″N and 105°46′36.18″E, and for VASI is 20°56′41.49″N, 105°49′37.67″E. The two sites are about 24 km apart from each other. Soybean rust was endemic in the vicinity of the research institutes. The climate at the location is tropical with more frequent rains and higher temperatures occurring during the middle of the year from May to October. Average rainfall ranges from 19 mm in January to 318 mm in August. Average temperatures range from 14 to 19 °C in January as a minimum to 29 to 33 °C in June as the maximum. Efforts were made to time experimental planting dates to promote soybean rust development and avoid the hotter periods of the year.

2.2. Soybean genotypes

Soybean germplasm accessions and check soybean genotypes tested in this study, along with maturity group (MG) and the field experiments that included them are listed in Table 1. Maturity groups of the accessions ranged from MG I to MG IX. Resistant and susceptible checks in the field tests included lines with the soybean rust resistance genes *Rpp1*, *Rpp1b*, *Rpp2*, *Rpp3*, *Rpp4*, and *Rpp5*, and the Vietnamese cultivars 'Cao Bang U8352', 'DT 2000', and 'Vang Ha Giang'. Susceptible checks included the USA cultivar 'Williams 82' and Vietnamese cultivar 'DT 12'. All seeds were obtained from the

Table 2
Field experiments conducted at the Vietnam Agriculture Science Institute (2005) and the Plant Protection Research Institute (2006–2009), Hanoi, Vietnam.

Experiment number	Number of soybean genotypes	Description	Planting date	Rust assessment dates[a]
1	63	Initial test of soybean rust resistance. Soybean genotypes were grouped according to plant maturity into three separate tests.	17 February, 2005	17 March to 14 April, 2005
2	16	Follow-up test of soybean genotypes with the highest rust resistance in Experiment 1.	10 October, 2005	15 November and 28 December, 2005
3	40	Follow-up test of resistant soybean genotypes tested in Experiment 1.	10 February, 2006	March to 24 April, 2006
4	11	Follow-up test of soybean genotypes with the highest rust resistance in Experiment 3.	24 February, 2007	4 April to 10 May, 2007
5	38	Comparison of known rust-resistant soybean genotypes with plant introductions found to have resistance at other locations.	24 October, 2008	28 November, 2008 to 14 January, 2009

[a] Soybean rust ratings were recorded during growth stages R3, R4, R5, and R6 for each soybean genotype within each experiment.

USDA Soybean Germplasm Collection, except for the Vietnamese cultivars that were obtained from the MARD.

2.3. Experimental design

Table 2 lists information on the five field experiments conducted in this study, including the experiment number, number of soybean genotypes, year conducted, and a brief description. Ten seeds of each accession were sown in a 1 m row with 60 and 10 cm spacing between and within rows, respectively. All experiments were arranged in a randomized complete block design (RCBD) with four replicated blocks within each test. The experimental unit was five plants in each test plot. All experiments were conducted during the normal soybean-growing season in Vietnam. Planting dates were chosen to synchronize the most receptive soybean developmental stages (V1–V3) with cooler temperatures conducive for soybean rust development.

Soybean rust susceptible cultivars DT 12 and 'V 74' were planted 4 weeks prior to the soybean test genotypes in border rows surrounding each experiment and were inoculated 2 weeks earlier than the test soybean genotypes to provide rust inoculum that would spread naturally to the test plots. In Experiment 1, genotypes were separated into three sub-experiments grouped by MG. All three groups of genotypes were inoculated on the same date, but were evaluated at different times according to soybean developmental stages. Experiments 2 through 4 were follow-up tests of genotypes that appeared rust-resistant in Experiment 1 to evaluate resistance performance across years. Experiment 5 was a test to compare known sources of resistance with germplasm accessions found to be resistant at other locations. Susceptible check genotypes were included in all of the tests.

2.4. Soybean rust inoculation

Soybean rust inoculum was collected from soybean fields near VASI each year for each test and was maintained on susceptible plants of the local soybean cultivars DT 12 and V 74 in screen houses. Because the inoculum collected was bulked, there was potential for a mixture of different soybean rust pathotypes included in the inoculum. Urediniospores were washed from infected leaves with water and filtered through a 53-μm nylon screen. Concentration of suspended urediniospores was quantified and standardized to 5×10^4 spores per ml with a hemocytometer. Plants were inoculated three times, at 1–2-week intervals, from growth stages V2 to R1 (Fehr et al., 1971) using a hand-pressured sprayer until runoff. To retain humidity, rows were covered for the first 24 h after inoculation by black plastic sheeting suspended above the canopy on bamboo stakes. The day prior to plant inoculation, furrows adjacent to the rows were filled with water. After inoculation, the furrows were filled daily until soybean rust lesions were observed. The experiments were furrow-irrigated throughout the remaining season as needed usually at 3–4 days intervals.

2.5. Soybean rust severity assessment

Soybean rust severity and lesion type were assessed on five marked randomly selected plants in each plot during growth stages R3, R4, R5, and R6 (Fehr et al., 1971). For Experiments 1 and 2, each marked plant on each assessment date was rated using a 1–4 scale where 1 = no symptoms, 2 = low number of lesions (1–100), 3 = moderate number of lesions (101–500), and 4 = high number of lesions (>500). The assessment was recorded for one leaflet of each plant at the lower canopy level often taking the lowest attached leaflet on the main stem. For Experiments 3, 4, and 5, a percentage scale was used based on: 0, 5, 10, 20, 30, 50, 75, and 100% of leaf area affected. Four mean severity ratings of five plants per plot, which was the experimental unit, taken during each of the four growth stages, were used to calculate the area under disease progress curve or AUDPC (Shanner and Finney, 1977) for each plot in each replication in each test. Lesion type was recorded as RB, TAN, intermediate (INT), or mixed (MX). Intermediate lesions had color, size and sporulation levels that appeared to be intermediate between RB or TAN lesions. Plants that clearly had both lesion types were classified as MX.

2.6. Statistical analysis

AUDPC data were analyzed by analysis of variance using Restricted Maximum Likelihood procedure in JMP 5 (SAS Institute, Cary, NC). Means were compared by Students' least significant difference (LSD) at $P = 0.05$. Fourteen soybean genotypes tested in Experiments 1, 2, and 3 were combined and analyzed by analysis of variance to determine if there was a significant genotype × experiment interaction.

3. Results

3.1. Experiment 1

AUDPC units differed ($P < 0.05$) among soybean genotypes within each of the three maturity groups (Table 3). AUDPC units of the cultivars bred for soybean rust resistance in Vietnam, Cao Bang U8352, DT 2000, and Vang Ha Giang, were among the lowest in the experiment. Germplasm accessions with the soybean rust resistance genes *Rpp1* (PI 200492) and *Rpp2* (PI 230970) did not differ from the resistant check Vang Ha Giang in the MG V–VII test, whereas the germplasm accessions with *Rpp3* (PI 462312) and *Rpp4* (PI 459025B) did not differ from the susceptible checks DT 12 (VIII–IX test) and Essex (V–VII test), respectively.

Table 3
Resistance of 63 soybean genotypes in three separate tests grouped according to cultivar maturity in field Experiment 1 at the Vietnam Agriculture Science Institute, Hanoi, Vietnam in 2005.

Soybean genotype	AUDPC[a]	Reaction type[b]
Maturity groups III–IV		
DT 2000[c]	42	RB
PI 561287A	56	TAN
PI 437323	58	RB
PI 549017	60	RB
PI 548178	61	TAN
PI 547878	63	TAN
Pana	70	TAN
PI 567565	70	RB
PI 068494	70	TAN
Ina	71	TAN
PI 091730	71	TAN
PI 083881	71	TAN
PI 385942	72	RB
PI 508269	74	TAN
PI 594250	75	TAN
PI 243524	75	TAN
PI 092560	76	TAN
PI 088452	80	TAN
PI 407730	80	TAN
PI 547875	80	TAN
Williams 82[d]	81	TAN
Rend	85	TAN
Mean	71	
LSD ($P = 0.05$)	10	
Maturity groups V–VI		
PI 200492 (*Rpp1*)	53	TAN
PI 427241	55	TAN
PI 429329	58	TAN
PI 398998	59	RB
PI 319525	59	TAN
Vang Ha Giang[c]	61	RB
PI 628859	63	TAN
PI 230970 (*Rpp2*)	65	RB
PI 165914	67	TAN
PI 548484	67	TAN
PI 561287B	69	TAN
PI 561381	71	TAN
PI 208437	73	RB
PI 548463	73	TAN
Bragg	75	TAN
PI 518759	76	TAN
PI 085089	78	TAN
PI 459025B (*Rpp4*)	78	RB
PI 417088	78	RB
Essex[d]	79	TAN
Lee 74	79	TAN
PI 459025F	80	TAN
PI 594172A	80	TAN
PI 424456	82	TAN
Mean	70	
LSD ($P = 0.05$)	10	
Maturity groups VII–VIII		
PI 594538A	41	RB
Cao Bang U8352[c]	45	RB
PI 340898A	52	TAN
PI 417089A	57	TAN
PI 240667A	59	TAN
PI 423972	59	RB
PI 434973A	62	TAN
PI 164885	64	TAN
Jupiter-R	66	TAN
Cook	67	TAN
PI 189402	70	TAN
PI 417317	70	TAN
PI 206258	74	TAN
PI 462312 (*Rpp3*)	79	TAN
DT 12[d]	80	TAN
Mean	63	
LSD ($P = 0.05$)	15	

[a] Soybean rust severity was assessed at soybean growth stages R3, R4, R5, and R6 (Fehr et al., 1971) at the lower canopy level of five plants with a 1–4 ordinal rating scale where 1 = no symptoms, 2 = low number of lesions (1–100), 3 = moderate number of lesions (101–500), and 4 = high number of lesions (>500). AUDPC values were calculated using the midpoint rule method (Shanner and Finney, 1977). Mean AUDPC of five soybean plants assessed in each plot were calculated and analyzed by ANOVA.
[b] RB = reddish-brown lesions and TAN = tan colored lesions.
[c] Soybean rust-resistant soybean cultivars.
[d] Soybean rust susceptible soybean cultivars.

The three resistant checks, Cao Bang U8352, DT 2000, and Vang Ha Giang, had RB soybean rust lesions while all susceptible checks had TAN lesions (Table 3). No immune or intermediate reaction types were observed in this experiment. Four out of 21 genotypes in MG III–IV, five out of 23 in MG V–VII, and two out of 13 in MG VIII–IX had RB lesions. PI 230970 with *Rpp2* and PI 459025B with *Rpp4* had RB lesions, whereas PI 200492 with *Rpp1*, and PI 462312 with *Rpp3* had TAN lesions. Soybean accessions that produced RB lesions also tended to result in lower AUDPC units.

3.2. Experiment 2

There were significant differences in AUDPC units among the 16 soybean genotypes evaluated in this experiment ($P < 0.05$). Ten soybean genotypes with low AUDPC units in Experiment 1 (Table 3) also had lower AUPDC units in this experiment (Table 4). Three of the genotypes, PI 230970 (*Rpp2*), PI 548463, and PI 594172A, had AUDPC units not significantly different ($P = 0.05$) from Cao Bang U8352, the Vietnamese soybean rust-resistant cultivar, which had the lowest AUDPC units among resistant checks in this follow-up test. An additional four soybean genotypes, PI 398998, PI 548484,

Table 4
Resistance of 16 soybean genotypes to soybean rust in field Experiment 2 at the Plant Protection Research Institute, Hanoi, Vietnam in 2006.

Soybean genotype	AUDPC[a]	Soybean rust reaction type[b]
PI 230970 (*Rpp2*)	41	RB
Cao Bang U8352[c]	42	RB
PI 548463	45	TAN
PI 594172A	45	TAN
DT 2000	48	RB
PI 398998	49	RB
PI 548484	49	TAN
PI 462312 (*Rpp3*)	50	TAN
PI 200492 (*Rpp1*)	52	TAN
Pana	53	TAN
PI 427241	53	TAN
PI 561287A	53	TAN
Jupiter-R	58	TAN
Williams 82[d]	60	TAN
PI 459025F	67	RB
PI 189402	71	TAN
Mean	52	
LSD ($P = 0.05$)	4	

[a] Soybean rust severity was assessed at soybean growth stages R3, R4, R5, and R6 (Fehr et al., 1971) at the lower canopy level of five plants with a 1–4 ordinal rating scale where 1 = no symptoms, 2 = low number of lesions (1–100), 3 = moderate number of lesions (101–500), and 4 = high number of lesions (>500). AUDPC values were calculated using the midpoint rule method (Shanner and Finney, 1977). Mean AUDPC of five soybean plants assessed in each plot were calculated and analyzed by ANOVA.
[b] RB = reddish-brown lesions and TAN = tan colored lesions.
[c] Soybean rust-resistant soybean cultivar.
[d] Soybean rust susceptible soybean cultivar.

Table 5
Resistance of 40 soybean genotypes to soybean rust in field Experiment 3 at the Plant Protection Research Institute, Hanoi, Vietnam in 2006.

Soybean genotype	AUDPC[a]	Soybean rust reaction type[b]
PI 437323	364	RB
PI 230970 (*Rpp2*)	367	RB
PI 549017	387	TAN
DT 2000[c]	388	RB
PI 398998	426	RB
Cao Bang U8352[c]	428	INT
Vang Ha Giang[c]	438	INT
PI 423972	492	INT
PI 459025B (*Rpp4*)	501	RB
PI 319525	507	TAN
PI 088452	514	TAN
PI 561287B	525	TAN
PI 518759	542	TAN
PI 594172A	549	TAN
PI 085089	557	TAN
Pana	559	TAN
PI 208437	567	TAN
PI 462312 (*Rpp3*)	604	TAN
PI 594538A (*Rpp1b*)	607	TAN
Rend	621	TAN
PI 417317	642	TAN
PI 548463	645	TAN
PI 548484	677	TAN
PI 567565	692	TAN
PI 200492 (*Rpp1*)	697	TAN
PI 417089A	704	TAN
PI 561287A	706	TAN
Bragg	726	TAN
Williams 82[d]	750	TAN
PI 068494	764	TAN
PI 407730	797	TAN
PI 427241	841	TAN
PI 240667A	877	TAN
PI 459025F	898	TAN
PI 429329	937	TAN
PI 628859	938	TAN
PI 164885	942	TAN
PI 189402	1024	TAN
Jupiter-R	1079	TAN
PI 206258	1400	TAN
Mean	667	
LSD (P = 0.05)	133	

[a] Soybean rust severity was assessed at soybean growth stages R3, R4, R5, and R6 (Fehr et al., 1971) at the lower canopy level of five plants with a percentage scale based on: 0, 5, 10, 20, 30, 50, 75, and 100% of leaf area affected. AUDPC values were calculated using the midpoint rule method (Shanner and Finney, 1977).
[b] RB = reddish-brown lesions and TAN = tan colored lesions, INT = lesions had the color, size and sporulation level between or not clearly RB or TAN.
[c] Soybean rust-resistant soybean cultivars.
[d] Soybean rust susceptible soybean cultivar.

PI 462312 (*Rpp3*), and PI 200492 (*Rpp1*), had AUDPC units that were not significantly different (P = 0.05) from the resistant check DT 2000. Three soybean genotypes, Jupiter-R, PI 459025F (*Rpp4*), and PI 189402, had AUDPC units not significantly different from Williams 82 in this experiment (Table 4). PI 200492 with *Rpp1*, PI 230970 with *Rpp2*, and PI 462312 with *Rpp3* soybean rust resistance genes had AUDPC that were not significantly different from DT 2000 (Table 4). Soybean rust reaction types were identical in this experiment (Table 4) as were observed in Experiment 1 on the genotypes (Table 3).

3.3. Experiment 3

There were significant (P < 0.05) differences in AUDPC units among the 40 soybean genotypes tested in this experiment. Nine soybean genotypes had AUDPC units that did not differ (P < 0.05) from the AUDPC units on the resistant check DT 2000 (Table 5). Fourteen genotypes had AUDPC units not different (P < 0.05) from the AUDPC units on Cao Bang U8352. PI 200970 with *Rpp2* had AUDPC units not different (P < 0.05) from either DT 2000 or Cao Bang U8352. In contrast with Experiment 1 (Table 3), PI 459025B (*Rpp4*) had AUDPC units not significantly different (P < 0.05) from both of the Vietnamese resistant checks (Table 5). There were 22 genotypes that had AUDPC units not significantly different (P < 0.05) or that were significantly higher (P < 0.05) than the susceptible check cultivar Williams 82 in this experiment (Table 5). PI 462312 (*Rpp3*) and PI 459025F had AUDPC units not significantly different (P < 0.05) from AUDPC units on Williams 82. AUDPC units on PI 200492 with *Rpp1* were also not different (P < 0.05) from AUDPC units on Williams 82, in contrast with results in both Experiments 1 (Table 3) and 2 (Table 4). Cao Bang U8352 and Vang Ha Giang had intermediate reaction type in this experiment, while they had RB lesions in Experiments 1 and 2. PI 423972 had an intermediate lesion type in this experiment but produced RB lesions in Experiment 1. PI 549017 and PI 567565 had RB lesions in Experiment 1 but had TAN lesions in Experiment 3.

Table 6
Resistance of 11 soybean genotypes to soybean rust in field Experiment 4 at the Plant Protection Research Institute, Hanoi, Vietnam in 2007.

Soybean genotype	AUDPC[a]	Soybean rust reaction type[b]
DT 2000[c]	250	RB
PI 437323	258	RB
PI 549017	273	TAN
PI 398998	300	RB
PI 459025B (*Rpp4*)	305	INT
PI 594538A (*Rpp1b*)	323	TAN
PI 423972	339	RB
PI 230970 (*Rpp2*)	339	INT
PI 567565	363	TAN
PI 208437	472	TAN
Williams 82[d]	480	TAN
Mean	337	
LSD (P = 0.05)	15	

[a] Soybean rust severity was assessed at soybean growth stages R3, R4, R5, and R6 (Fehr et al., 1971) at the lower canopy level of five plants with a percentage scale based on: 0, 5, 10, 20, 30, 50, 75, and 100% of leaf area affected. AUDPC values were calculated using the midpoint rule method (Shanner and Finney, 1977).
[b] RB = reddish-brown lesions and TAN = tan colored lesions, INT = lesions had the color, size and sporulation level between or not clearly RB or TAN.
[c] Local soybean rust-resistant soybean cultivar.
[d] Soybean rust susceptible soybean cultivar.

3.4. Experiment 4

PI 437323 and PI 549017 had an AUDPC unit that did not differ from DT 2000, the resistant check (Table 6) while PI 208437 did not differ from Williams 82, the susceptible check. The other seven entries had intermediate AUDPC units, including PI 459025B (*Rpp4*) and PI 230970 (*Rpp2*), compared with DT 2000 and Williams 82.

PI 567564 and PI 208437 produced TAN lesions in this experiment (Table 6), and in Experiment 3 (Table 5), but RB lesions in Experiment 1 (Table 3). PI 549017 had TAN lesions in this experiment (Table 6) but had RB lesions in Experiment 1 (Table 3). PI 423972 had an intermediate lesion in this experiment (Table 6), but had RB lesions in Experiments 1 (Table 3) and 3 (Table 5). PI 459025B (*Rpp4*) had intermediate lesions in this experiment (Table 6) but produced RB lesions in both Experiments 1 (Table 3) and 3 (Table 5). PI 230970 (*Rpp2*) produced intermediate lesions in this experiment (Table 6) but produced RB lesions in Experiments 1 (Table 3), 2 (Table 4), and 3 (Table 5).

3.5. Experiment 5

There were 10 genotypes with AUDPC units similar to PI 459025B (*Rpp4*), which had the lowest AUDPC value and produced RB lesions (Table 7). These 10 included PI 200492 (*Rpp1*), PI 462312

(*Rpp3*), and PI 594538A (*Rpp1b*). The other seven genotypes with low AUDPC units had mostly RB lesions, and had not been tested in the previous experiments. PI 200526 (*Rpp5*) was among the genotypes with the highest AUDPC, and it produced TAN lesions.

3.6. Overall summary

The sources of *Rpp1-5* were not consistent from test to test in their AUDPC units or in the reaction type (Table 8). For example, PI 200492 (*Rpp1*) had the lowest AUDPC units in Experiments 1 and 5, but had intermediate AUDPC units in the other experiments and varied in reaction type among experiments. PI 230970 (*Rpp2*) had the lowest AUDPC in three of the five experiments with RB lesions in all experiments but one where the lesion type was classified as an intermediate reaction.

The resistant check, DT 2000, had among the lowest AUDPC ratings in three of the four experiments, and produced RB lesions in each of four experiments (Table 8). PI 398998 had among the lowest AUDPC units in one experiment, lower than average AUDPC units in three experiments, and consistently produced RB lesions. PI 437323 was among the genotypes with the lowest AUDPC units in two of three experiments and consistently produced RB lesions. PI 549017 was among the soybean genotypes with the lowest AUDPC in one of three experiments, but produced RB lesions in one test and TAN lesions in two other tests.

A significant experiment × genotype interaction ($P < 0.001$) was found when the AUDPC data of 14 soybean genotypes tested in Experiments 1, 2, and 3 were combined and analyzed (Table 9). This result indicated that the response of the genotypes to soybean rust was dependent on the experiment and therefore the time the test was conducted.

4. Discussion

The development of soybean cultivars with resistance to soybean rust is an important component in an integrated program to manage the disease. The first step in developing new resistant cultivars is the selection of sources with effective soybean rust resistance. With high virulence diversity known among *P. pachyrhizi* isolates (Bonde et al., 2006; Pham et al., 2009), the effectiveness of soybean rust resistance may vary across locations and must be verified in targeted geographic areas through field testing over several years. It was evident in our study that the virulence of the field population of *P. pachyrhizi* was not constant based on

Table 7
Resistance of 38 soybean genotypes to soybean rust in field Experiment 5 at the Plant Protection Research Institute, Hanoi, Vietnam in 2008–2009.

Soybean genotype	AUDPC[a]	Reaction type[b]
PI 459025B (*Rpp4*)	641	RB
PI 594538A (*Rpp1b*)	732	MX
PI 615437	737	MX
PI 561356	766	RB
PI 462312 (*Rpp3*)	798	RB
PI 561377	805	MX
PI 594767A	809	RB
PI 200492 (*Rpp1*)	817	RB
PI 567104B	820	RB
PI 081765	833	RB
PI 417125	837	MX
PI 506947	848	MX
PI 567046A	869	RB
PI 379621	874	MX
PI 230970 (*Rpp2*)	874	RB
PI 567034	885	MX
PI 506695	897	TAN
PI 417116	903	TAN
PI 417120	904	TAN
PI 200478	918	TAN
PI 203398	942	TAN
PI 594754	972	MX
PI 606405	982	TAN
PI 417904	1003	TAN
PI 587886	1010	TAN
Williams 82[c]	1020	TAN
PI 605885B	1107	TAN
PI 200456	1108	TAN
PI 417089A	1109	TAN
PI 567024	1118	TAN
PI 605791A	1139	TAN
PI 200526 (*Rpp5*)	1189	TAN
PI 594172A	1208	TAN
PI 379618	1263	TAN
PI 417115	1268	TAN
PI 567031B	1275	TAN
PI 605773	1325	TAN
PI 605829	1333	TAN
Mean	966	
LSD ($P = 0.05$)	220	

[a] Soybean rust severity was assessed at soybean growth stages R3, R4, R5, and R6 (Fehr et al., 1971) at the lower canopy level of five plants with a percentage scale based on: 0, 5, 10, 20, 30, 50, 75, and 100% of leaf area affected. AUDPC values were calculated using the midpoint rule method (Shanner and Finney, 1977).
[b] RB = reddish-brown lesions, TAN = tan colored lesions, INT = lesions had the color, size and sporulation level between or not clearly RB or TAN, MX = mixed RB/TAN on the same plants.
[c] Soybean rust susceptible soybean cultivar.

Table 8
Summary of soybean rust area under disease progress curve values (AUDPC) and reaction type of known sources of resistance, a selected resistant cultivar in Vietnam, three plant introductions, and a susceptible cultivar from five experiments from 2005 to 2009 at the Plant Protection Research Institute, Hanoi, Vietnam.

Genotype	Experiment				
	1	2	3	4	5
PI 200492 (*Rpp1*)	53[a]/TAN	52/TAN	697/TAN	–	817[a]/RB
PI 594538A (*Rpp1b*)	41[a]/RB	–	607/TAN	323/TAN	732[a]/M
PI 230970 (*Rpp2*)	65/RB	41[a]/RB	367[a]/RB	339/INT	874/RB
PI 462312 (*Rpp3*)	79[b]/TAN	50/TAN	604/TAN	–	798[a]/RB
PI 459025B (*Rpp4*)	78[b]/RB	–	501/RB	305/INT	641[a]/RB
DT 2000	42[a]/RB	48/RB	388[a]/RB	250[a]/RB	–
PI 398998	59[a]/RB	49/RB	426[a]/RB	300/RB	–
PI 437323	58/RB	–	354[a]/RB	258[a]/RB	–
PI 549017	60/RB	–	387[a]/TAN	273/TAN	–
Williams 82	81/TAN	60/TAN	750/TAN	480/TAN	1020/TAN
Range (mean)	41-([c])-85	41-(52)-71	364-(667)-1400	250-(337)-480	641-(966)-1333
LSD ($P = 0.05$)	[d]	4	133	15	220

[a] AUDPC not significantly different ($P = 0.05$) from the lowest value in the test.
[b] AUDPC not significantly different ($P = 0.05$) from the highest value in the test.
[c] Mean AUDPC for each test of soybean genotypes grouped by plant maturity were 71 for the MG III–IV test, 70 for the MG V–VI test, and 63 for the MG VII–VIII test (Table 3).
[d] LSD was 10 for the MG III–IV test, 10 for the MG V–VI test, and 15 for the MG VII–VIII test (Table 3).

Table 9
Analysis of variance of AUDPC for 14 soybean genotypes tested in Experiments 1, 2, and 3.

Source	df	F	$P > F$
Genotype[a]	13	1.37	0.27
Experiment	2	1830	<0.001
Block (experiment)	6	1.79	0.24
Experiment × genotype	26	6.49	<0.001

[a] Soybean genotypes included in the analysis were Cao Bang, DT 2000, Jupiter-R, Pana, PI 189402, PI 200492, PI 230970, PI 398998, PI 427241, PI 462312, PI 548463, PI 548484, PI 561287A, and PI 594172A.

changes of reaction types and severity for some genotypes over different experiments (Table 3). However, there were some consistencies among the genotypes in reaction type and disease severity. For example, DT 2000, known to be resistant in Vietnam, produced RB lesions and low AUDPC units in all experiments. This cultivar was imported into Vietnam from the Asian Vegetable Research and Development Center (AVRDC) in 1997 and released for commercial use in 2001. The AUDPC of two other resistant checks, Cao Bang U8352 and Vang Ha Giang, was similar to that of DT 2000 in the experiments they were in together. Williams 82, a public cultivar from the USA, often is used as a soybean rust susceptible check since it produces TAN lesions to numerous *P. pachyrhizi* isolates (Pham et al., 2009), and the Vietnamese cultivar DT 12, also known to be susceptible, consistently produced TAN lesions with high AUDPC. Since differences in AUDPC values were found among the soybean genotypes in all five of the field experiments, this provides the basis for separating out resistant and susceptible genotypes. Three soybean genotypes, PI 398998, PI 437323, and PI 549017, had soybean rust resistance comparable with Vietnamese rust-resistant cultivars in many of the experiments. These three genotypes were previously short-listed as sources of resistance with high potential after testing in Fort Detrick (Miles et al., 2006). In that study, PI 398998 produced RB lesions, PI 437323 produced both RB and TAN, and PI 549017 had TAN lesions but low rust severity. In addition, PI 437323 produced RB lesions to two and TAN lesions to eight *P. pachyrhizi* isolates, respectively (Pham et al., 2009). These genotypes may have resistance genes not present in the Vietnamese cultivars and could be valuable sources of resistance for the development of new, rust-resistant cultivars for production in Vietnam or other locations with a soybean rust population having a similar virulence spectra. These genetic resources may be useful for host plant resistance studies and breeding soybeans for rust resistance in Vietnam and other locations like Brazil and the United States that have more recently been inundated with soybean rust. Genetic allelism tests and controlled resistance tests with soybean rust pathotypes that have virulence defined on a set of differential soybean genotypes are required to determine the uniqueness of resistance genes in the genotypes and their spectra of effectiveness against different soybean rust pathotypes.

Several sources of resistance tested in Vietnam in this study were also previously reported to have high levels of resistance in other locations including PI 567104B, PI 587866, and PI 594754 in Paraguay (Miles et al., 2008), and PI 417089A and PI 594538A in Nigeria (Twizeyimana et al., 2008). In our trial in Vietnam, PI 567104B and PI 594538A (*Rpp1b*) had among the lowest AUDPC units in many of the experiments, while PI 417089A, PI 587866, and PI 594754 were not different in AUDPC units from Williams 82. In another study, PI 417089A was reported to have high levels of rust resistance while PI 594538A produced mostly TAN lesions when challenged with six purified USA-collected isolates (Paul and Hartman, 2009).

None of the soybean rust resistance genes *Rpp1*, *Rpp1b*, *Rpp2*, *Rpp3*, or *Rpp4* appeared to provide as consistently strong resistance to soybean rust as the Vietnamese cultivar DT 2000 in this study; however, among the known resistance genes, resistance expressed by *Rpp2* appeared to be the most consistent. In other studies, these single resistance gene sources have had mixed results, more often with lower levels of resistance reported in field studies outside the USA (Miles et al., 2008; Twizeyimana et al., 2008). When challenged with USA-collected isolates, the resistance expression has also been mixed with the sources of resistance containing *Rpp1* and *Rpp3* showing high levels of resistance in one study (Paul and Hartman, 2009), but not in another study (Pham et al., 2009), and the sources of resistance containing *Rpp2* and *Rpp4* showing high levels of resistance in one study (Pham et al., 2009), but not in another study (Paul and Hartman, 2009). Neither of these studies used the same isolates of *P. pachyrhizi*. This emphasizes the need to develop a common differential set of soybean lines and characterized "tester" isolates to make broader comparisons about isolates and sources of resistance.

Overall, results of this study indicated new, potentially useful sources of resistance to soybean rust that may be valuable to soybean breeders in Vietnam and other locations like Brazil and the United States that have more recently been inundated with soybean rust. Follow-up studies are recommended to identify and map the genomic locations of the resistance genes present in the identified resistance sources and determine their effectiveness against specific, characterized *P. pachyrhizi* pathotypes.

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